LARS Information Note 111472

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The Laboratory for Applications of Remote Sensing

Purdue University
West Lafayette, Indiana

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ABSTRACT

Reflectance, transmittance and absorptance spectral of "normal" and six types of nutrient-deficient (N, P, K, S, Mg, and Ca) maize (Zea mays L.) leaves were analyzed at 30 selected wavelengths from 500-2600 nm. The analysis of variance showed significant differences in reflectance, transmittance and absorptance in the visible wavelengths among leaf numbers 3, 4, and 5, among the seven treatments, and among the interactions of leaf number and treatments. In the infrared wavelengths only treatments produced significant differences.

The chlorophyll content of leaves was reduced in all nutrient-deficient treatments. Percent moisture was increased in S-, Mg-, and N-deficiencies. Positive correlations were obtained ($r = 0.7$) between moisture content and percent absorption at both 1450 and 1930 nm. Polynomial regression analysis of leaf thickness and leaf moisture content showed that these two variables were significantly and directly related ($R = 0.894$). Leaves from the P- and Ca-deficient plants absorbed less energy in the near infrared than the normal plants; S-, Mg-, K-, and N-deficient leaves absorbed more than the normal.

Leaf thermograms were prepared on normal and S- and N-deficient leaves. Both S- and N-deficient leaves had higher temperatures than normal maize leaves.

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[1]Journal Paper No. 4839, Purdue University Agricultural Experiment Station. Supported by: U.S.D.A. Contract #12-14-100-10292 (20), NASA Grant NGR 15-005-112, N.S.F. Grant GB5701, National Institute of General Medical Science Career Grant K6-21839, and Training Grant GM01392.

[2]Research Agronomist, Research Associate, Former Graduate Assistant, Professor of Biology, and Associate Professor of Agronomy.

Additional Key Words: Reflectance, Absorptance, Transmittance, Leaf Chlorophyll Content, Leaf Moisture Content, Leaf Thickness, Leaf Thermogram.

INTRODUCTION

The characteristics of plant spectral reflectance and transmittance are functions of leaf geometry, morphology, physiology, and biochemistry. They are also influenced by soil and climatic conditions (4) and nutrient status (3). Excess or deficiency of an essential element also may cause visible abnormalities in pigmentation, size and shape of leaves and the appearance of various other symptoms.

Previous studies in the general area of leaf spectral reflectance and transmittance include the effect of physiological age (7), water content (10,18), osmotic stress and salinity (8), pigment composition (2), the relation of cell structure upon individual leaf spectra [6, (Gausman, H. W., W. A. Allen, C. L. Wiegand, D. E. Escobar, R. R. Rodriguez and A. J. Richardson. The leaf mesophylls of twenty crops, their spectra and optical and geometrical parameters. Annual Report, Weslaco, Texas.), 17], and plant nutrient stress (11, 16, 19).

The objective of this research was to study the spectral characteristics of normal and nutrient-deficient (-N, -P, -K, -S, -Ca, -Mg) maize (*Zea mays* L.) leaves. The results of this study should provide basic knowledge for the interpretation of spectral measurements from air- and space-borne sensors.

MATERIALS AND METHODS

Maize plants were grown in the greenhouse in seven different nutrient culture solutions as follows: normal, -N, -P, -K, -Ca -Mg, and -S using Hoagland and Arnon's nutrient solutions (9). EDTA-chelated Fe was substituted for $Fe_2(SO_4)_3$ as the source of iron. Inert volcanic glass called "Krum" was used for root support (obtained from the Silbrico Corp., 6300 River Road, Hodgkins, Illinois 60825).[3] Randomized complete block design with five replicates was used.

Eight weeks after plant emergence, leaf sections 10-12 cm long from the broadest part of every leaf from five plants of normal and the nutrient-deficient treatments were removed for analysis. Leaves

[3] Use of a company or product name in this paper does not imply its approval or recommendation to the exclusion of others that may also be suitable.

were numbered consecutively from base to tip. Leaf number 3, 4, and 5 were sampled because they are present in plants of all treatments. Total reflectance and transmittance spectra were obtained over the 500-2600 nm wavelength interval with a Beckman DK-2A spectroreflectometer using barium sulfate as standard.

Fresh weight and area of each leaf section were determined immediately after completing the DK-2A spectral measurements. From these data, leaf thicknesses (defined in this paper as weight/unit area) were calculated. The dry weights of these leaves were also determined after drying leaf sections in an oven at 100°C for 48 hours. The difference between fresh and dry weights was used to calculate percent moisture on a dry weight basis.

The average chlorophyll content was determined from an 80% acetone extract of a 5g leaf sample pooled from 5-6 fresh leaves of the same chronological age (1). Chlorophyll values are reported here on a fresh weight basis (12).

The DK-2A graphs for both reflectance and transmittance spectra were reduced in a manner similar to that reported by Johannsen (10). All the data, after correction to give absolute values, were punched on cards and transferred to magnetic tape to facilitate handling and storage. The 30 wavelengths selected were: 500, 530, 600, 640, 700, 740, 830, 900, 940, 1000, 1100, 1160, 1200, 1300, 1400, 1430, 1450, 1500, 1550, 1600, 1700, 1770, 1800, 1930, 2000, 2100, 2180, 2300, 2400, and 2600 nm.

Absorptance was also calculated from the absolute values as:

Absorptance = 100% - (% reflectance + % transmittance)

Reflectance, transmittance, and calculated absorptance data for the seven nutrient treatments of leaves, 3, 4, and 5 were analyzed using analysis of variance, and the Scheffe' test (15) for multiple comparisons. Fourteen selected wavelengths (530, 640, 830, 940, 1100, 1200, 1400, 1430, 1450, 1550, 1700, 1930, 2000, and 2180 nm) out of the 30 recorded were used in this analysis.

A thermoscope[4] was used to obtain thermograms of three leaves from each of the normal, N-deficient, and S-deficient plants at the age of 8 weeks. This instrument measures the emitted energy in the spectral range of 6-14 μm from which instant temperature maps or thermograms are produced on a television-type screen and photographed (Texas Instruments, 1970, An introduction to medical thermography. Texas Instrument Incorporated). The scanning time used to develop the thermogram was 4.5 seconds. A "styrofoam" surface was used as a background for the maize leaves. Three densitometer readings were made at the center of the polaroid negative, and the temperature values were determined from a calibrated density scale.

[4] The thermogram and the densitometry measurement were carried out by courtesy of Texas Instruments, Inc., Stafford, Texas.

RESULTS

Results from the analysis of variance for the reflectance, transmittance, and absorptance data at 530 nm and 640 nm show significant differences (P = .01) among leaf numbers 3, 4, and 5, among the seven nutrient treatments, and among the interactions of leaves and treatments. Thus, the spectral variation in these visible wavelengths was influenced by the physiological age in leaves as well as the plant nutrient deficiencies.

The Scheffe' test results for absorptance data at 530 and 640 nm are summarized by tabular and graphic form (Table 1, Figure 1). Some of the calculated absorptance variation among the leaves at these wavelengths may be due to small differences in placement of the leaf sample in the spectroreflectometer for both transmittance and reflectance measurements.

The highest absorptance values were obtained in all cases for the leaves of normal, Ca- and P-deficient plants. This is in agreement with the chlorophyll content of these leaves in a direct relationship noted from Table 2. The Scheffe' multiple comparison test results (Table 1) for both wavelengths also indicate that the means of normal, Ca-, and P-treatments were significantly (P = .05) greater than the means of N-, K-, Mg-, and S-deficient leaves. Other comparisons of means were included and in general the results show the effect of nutrient deficiencies on the spectral characteristics of the maize throughout the visible region of the spectrum.

All nutrient-deficient plants contained less chlorophyll than the control plants (Table 2). These results indicate that chlorophyll has a dominant influence on spectral variations in the visible region of the spectrum.

Results of the analysis of variance for the spectral reflectance, transmittance, and absorptance of the remaining twelve wavelengths at the near and the infrared regions were found to be very similar. In all cases only the variation due to treatments was found to be significant (P = 0.01).

It is important to emphasize that leaf chronological age did not influence the spectra (750-2600 nm) of leaves having the same nutrient treatment.

The Scheffe' test for absorptance shows that only Mg-deficiency is significantly (P = .05) greater than P-deficiency at both 830 and 940 nm wavelengths but no significant differences were shown for other deficiencies. Also, significant differences were not obtained among all seven treatments at 1100 and 1200 nm wavelengths.

The Scheffe' test results show that S-deficient leaves had the highest level of absorptance among all deficiencies at wavelengths 1400, 1430, 1450, 1550, 1700, 1930, 2000 and 2180 nm; nitrogen and Mg-deficiencies showed second highest absorptance. These results correspond to the deficiencies with the lowest leaf moisture content shown in Table 2.

The average absolute values of the spectral reflectance and of the transmittance of leaves 3, 4, and 5 with each of the seven treatments were plotted separately for the wavelengths between 500-2600 nm (Figures 2 and 3). The area between percent transmittance and percent reflectance of these two figures is percent absorptance.

Leaf thickness versus leaf moisture content were plotted (Figure 5). Polynomial regression analysis was used to study the relationship between these two variables. A second degree polynomial was fitted to the data with an R^2 value of 0.80.

DISCUSSION

Solar radiation in the wavelength range from 500-2600 nm which reaches the earth may be absorbed, transmitted and reflected by plant leaves (4).

The spectral characteristics of the leaf in the visible region of the spectrum (500-750 nm) are associated mainly with leaf pigments (4, 14). Benedict and Swindler (2), working with soybeans and citrus, and Thomas and Oerther (19), working with sweet peppers, found an inverse relationship between reflectance and chlorophyll content.

The metabolic disturbances resulting from nutrient deficiencies of maize in this experiment led to a reduction of leaf chlorophyll content and consequently to an alteration of leaf color, reflectivity, and transmittance. These conditions are clearly illustrated (Figures 2 and 3). N-deficient maize has the least amount of chlorophyll and is followed in order of increasing chlorophyll content by -S, -Mg, -K, -Ca, -P, and normal maize plants (Table 1).

Reflectance and transmittance of the leaf in the near infrared region (750-1300 nm) is generally associated with leaf structure and morphology (6, (Gausman, H. W., W. A. Allen, C. L. Wiegand, D. E. Escobar, R. R. Rodriguez and A. J. Richardson. 1971. The leaf mesophylls of twenty crops, their spectra and optical and geometrical parameters. Annual Report, Weslaco, Texas.), 17). In young or immature leaves low reflectance and high transmittance is expected because cells are small and there are few intercellular spaces. As the leaf matures, differentiation into palisade and spongy mesophyll layers becomes more pronounced, intercellular spaces and vacuoles increase in size, and consequently reflectance increases. However, in monocots like maize there is no distinct organization of the mesophyll into palisade and spongy mesophyll layers (17). Myers et al. (13) have associated the severity of nitrogen-deficient sweet pepper leaves with increased near infrared reflectance. They attribute this increase in reflectance to smaller and fewer cells within the leaf. In the N deficient maize leaves of this study, cells were smaller and chloroplasts fewer in number than in the control (A. H. Al-Abbas, unpublished data). Similar results were obtained with S- and Mg-deficient maize leaves.

Among all treatments the K-deficient leaves gave the highest reflectance and had the lowest leaf thickness and least leaf moisture content. In addition to being related to cell size and altered structure, reflectance seems to be related closely with leaf thickness and moisture content.

It is interesting to note (Table 3) that the percent absorptance of -P and -Ca leaves at the wavelengths 830, 940 and 1100 nm was considerably lower than the absorptance value for the normal plant. Conversely, the absorptance of the -S, -Mg, -K, and -N leaves was much higher than that of the control. The low energy absorptance is associated with those treatments which have a high chloroplast number or a high chlorophyll content. The higher incident energy absorptance at these wavelengths by -S, -Mg, -K, and -N treatments may result from a higher heat content within the leaves and they may have a substantially higher temperature than do control, P-deficient and Ca-deficient plants. Results reported in this paper (Fig. 4) show this to be the case with -S and -N maize leaves. The low absorptance function of this region (near-infrared) will reduce the incident solar energy absorbed by the leaf and consequently protect the plant pigments from denaturation. However, the abnormality resulting from these nutrient deficiencies may affect stomatal development and transpiration which may lead to increased leaf temperature (3, 16).

The amount and spectral distributions of energy a leaf radiates is dependent upon its temperature (3). The thermogram (Fig. 3) illustrates the variation in temperature between normal, S-deficient, and N-deficient maize leaves. The average densitometry readings translated to temperature values (Table 4) confirm the visual observation from the thermogram which indicates that N-deficient leaves are warmer by 0.9°C and S-deficient leaves by 0.4°C than normal leaves. Gates (3) found an increase of 0.5°-1.5°C in K-deficient sugarcane leaves compared to normal ones when both were exposed simultaneously to sunlight.

The spectral reflectance and transmittance in the wavelength interval between 1300-2600 nm is related mainly to leaf water content (14). Although the main water absorption region is between

2600 and 2800 nm, liquid water affected reflectance strongly in the 1450 and 1930 nm bands which were significantly ($P = .01$) related to relative turgidity or water content of cotton leaves. Gausman et al. (7) obtained a positive correlation between cotton leaf water content and the absorptance coefficient (K) at 1950 nm. An examination of the water content of the various nutrient-deficient maize leaves in this study showed a positive correlation ($r = 0.7$) between the water content and percent absorptance at 1450 and 1930 nm wavelengths where both reflectance and transmittance are inversely proportional to leaf water content.

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Table 1. Scheffe' multiple comparison test results for absorptance of maize leaves 3, 4, and 5.

Wavelength	Leaf # 3	Leaf # 4	Leaf # 5
530 nm	Normal, -Ca >*-N, -K, -S -N, -P, -Mg>* -S	Norm., -P, -Ca>* -N, -K, -Mg, -S	Norm., -Ca>* -N, -Mg, -S -P >* -N, -S -K > -N
640 nm	Norm. >* -N, -P, -K, -Mg, -S -Ca >* -P, -K, -Mg, -S -P >* -Mg	Norm. >* -N, -K, -Mg, -S -Ca >* -K, -Mg, -S -P >* -K, -Mg	Norm. >* -N, -K, -Mg, -S -P, -Ca >* -N

>* = significantly greater than (P = .05).

Table 2. Average values of chlorophyll content, moisture content, and leaf thickness for normal and the six nutrient-deficient treatments of maize leaves.*

Nutrient Treatments	Chlorophyll Content mg/gm fresh weight	Leaf Thickness Expressed as mg/cm^2	Percent Moisture
Control	1.16	9.2	68.5
-P	1.02	4.0	49.7
-Ca	0.87	6.7	58.2
-K	0.63	4.7	46.4
-S	0.46	11.0	82.4
-Mg	0.43	9.0	73.2
-N	0.21	9.4	72.7

*Average values for leaves 3, 4, and 5.

Table 3. Average Percent Absorptance for Normal and Six Nutrient-Deficient Treatments of Maize Leaves.

Treatment	% Absorptance*		
	830 nm	940 nm	1100 nm
Control	2.37	2.92	3.66
-P	0.92	1.02	1.21
-Ca	1.71	1.40	2.10
-K	5.28	5.23	4.74
-S	5.57	5.74	7.11
-Mg	7.29	7.19	6.83
-N	4.72	4.91	5.50

*Average values for leaves 3, 4 and 5.

Table 4. Maize leaf temperature in degrees centigrade as calibrated from thermogram density.

Trial	Leaf Treatments - Temp.°C								
	Control			-S			-N		
	1	2	3	1	2	3	1	2	3
1	27.4	26.8	27.0	26.8	27.4	28.0	28.5	28.0	28.1
2	27.2	27.0	27.2	27.6	27.3	28.2	28.5	27.7	28.1
3	20.1	27.0	27.6	27.2	27.2	27.7	27.8	27.9	28.0
Avg.	27.2	26.9	27.2	27.2	27.3	28.0	28.2	27.9	28.1
Mean		27.1			27.5			28.1	

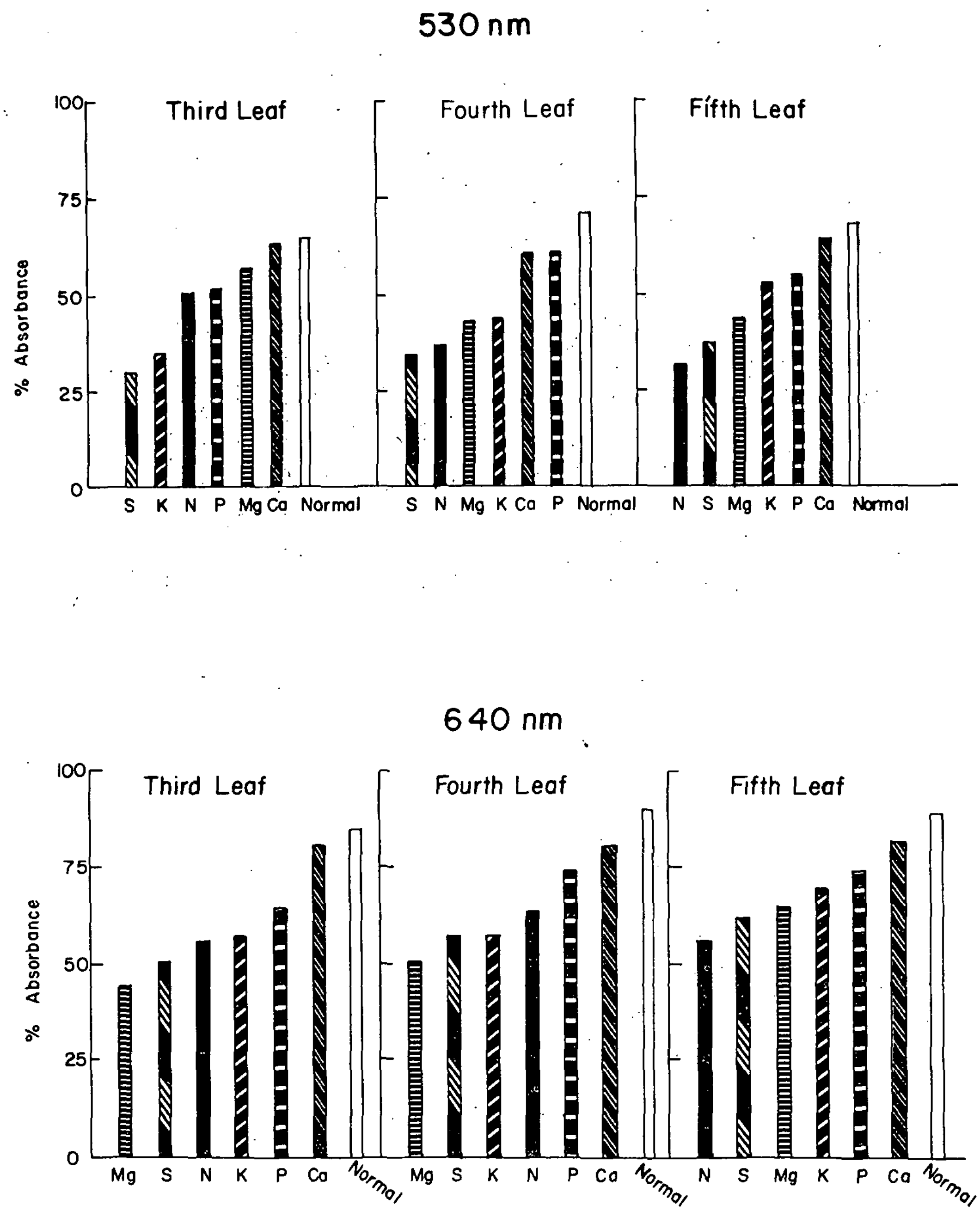

Figure 1. The spectral absorptance for N-, P-, K-, Ca-, Mg-, and S-deficient maize leaves 3, 4, and 5 at 530 nm and 640 nm wavelengths.

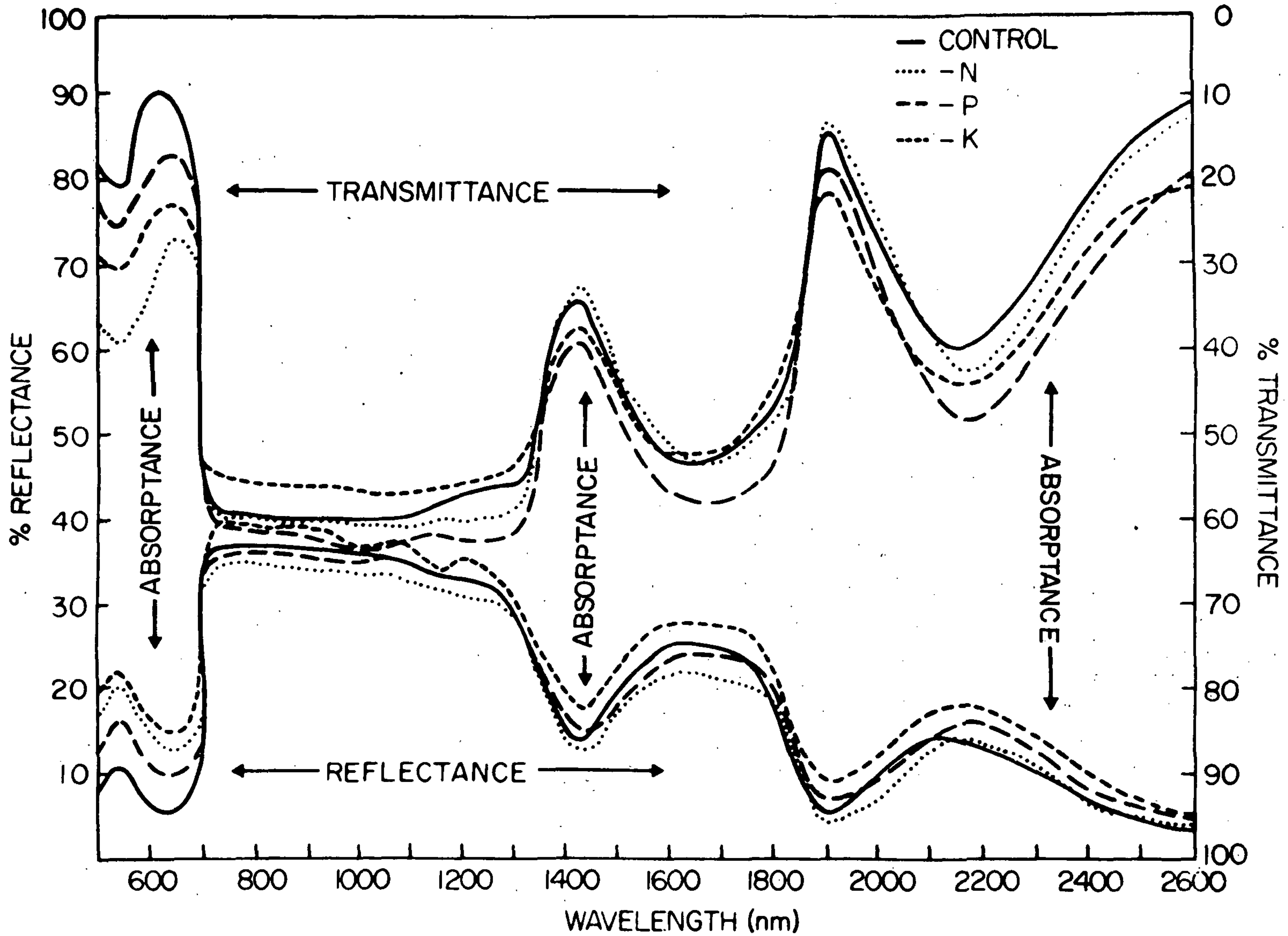

Figure 2. The relationship between the absolute spectral reflectance, transmittance, and absorptance for normal, N-, P-, and K-deficient maize leaves between 500-2600 nm.

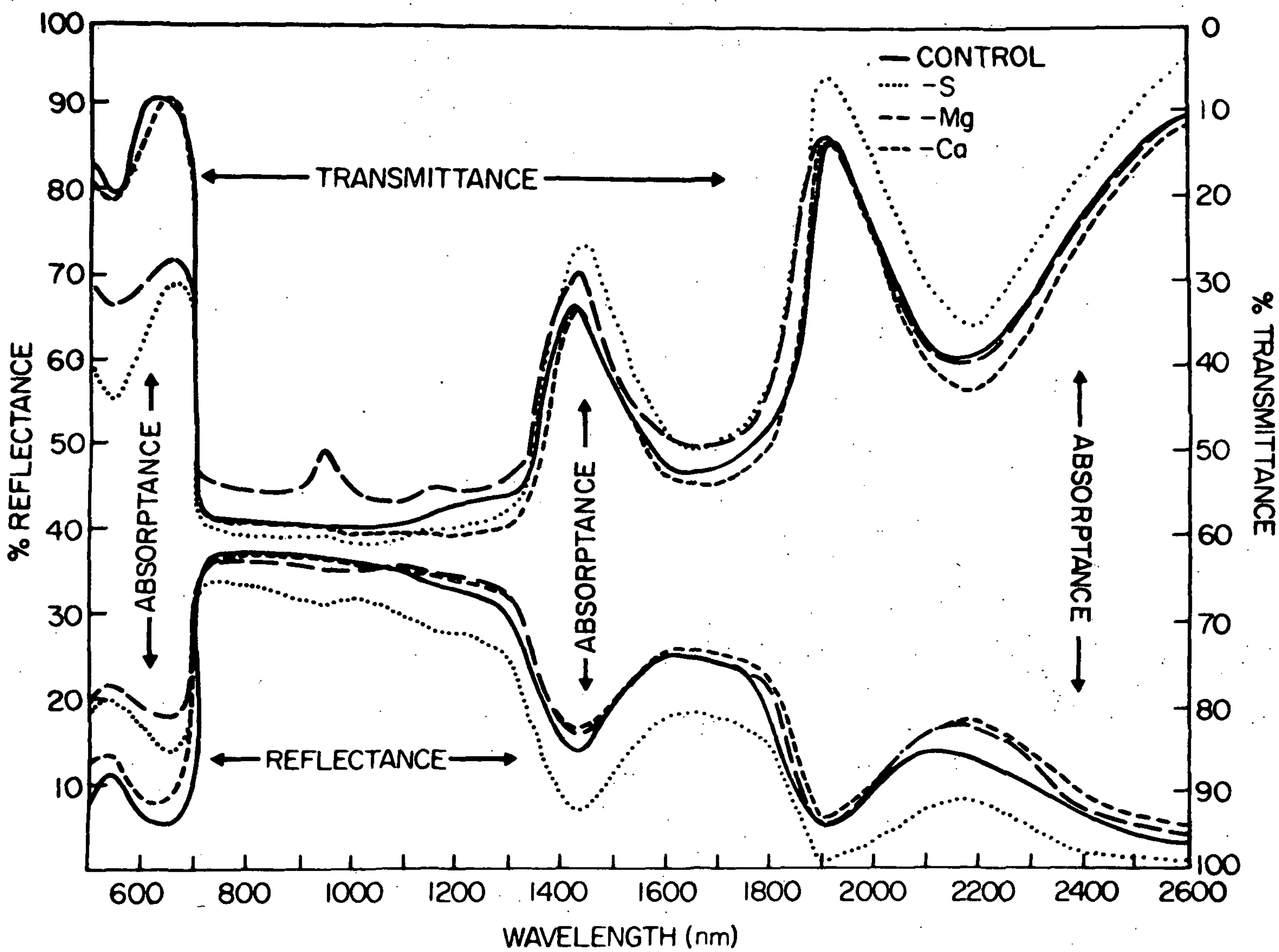

Figure 3. The relationship between the absolute spectral reflectance, transmittance, and absorptance for control, Ca-, Mg-, and S-deficient maize leaves between 500-2600 nm.

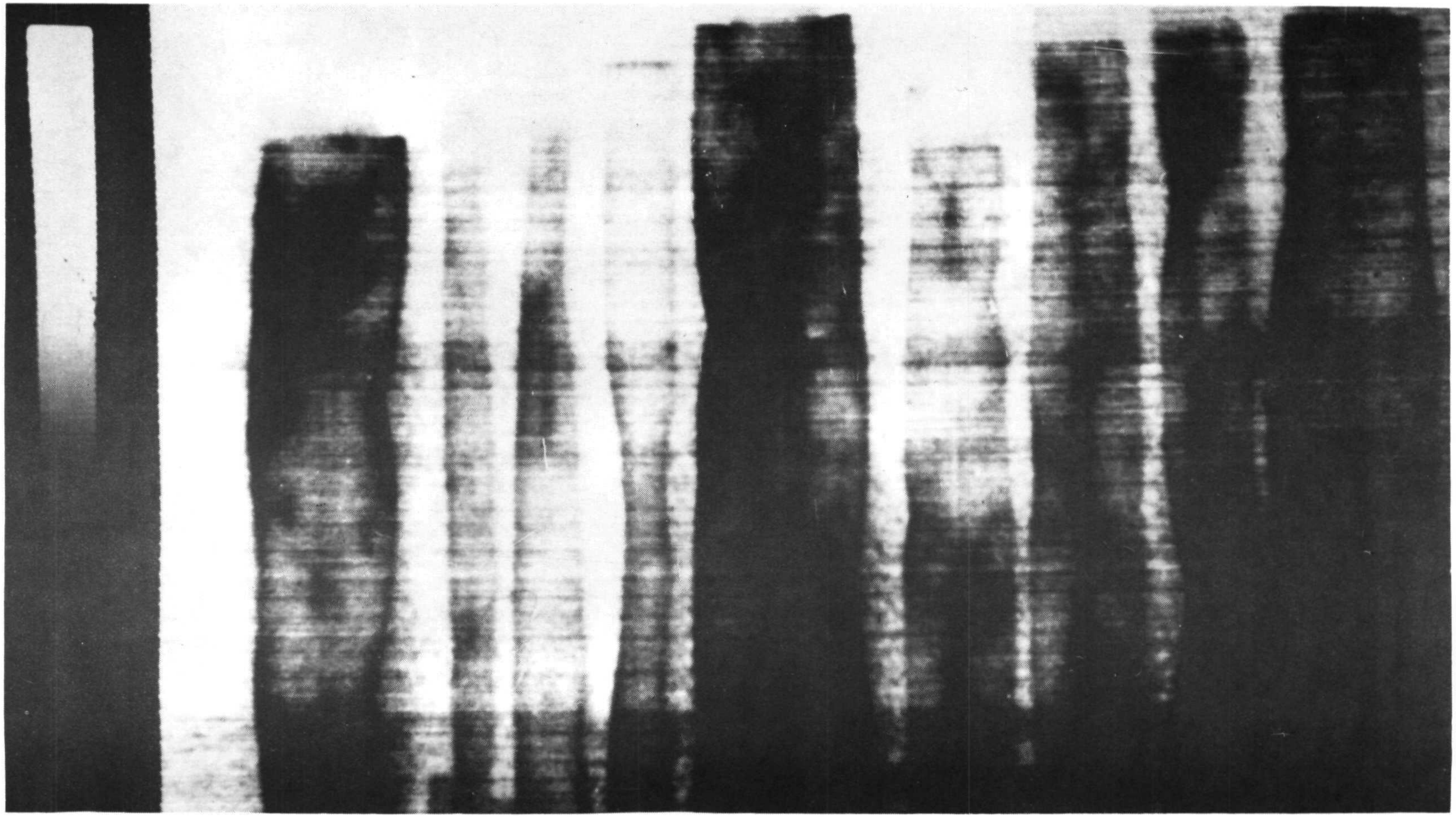

Figure 4. A thermogram of normal, S- and N-deficient maize leaves. From left to right, normal leaves are #1, 5 and 9; S-deficient #2, 3, and 4; N-deficient #6, 7 and 8.

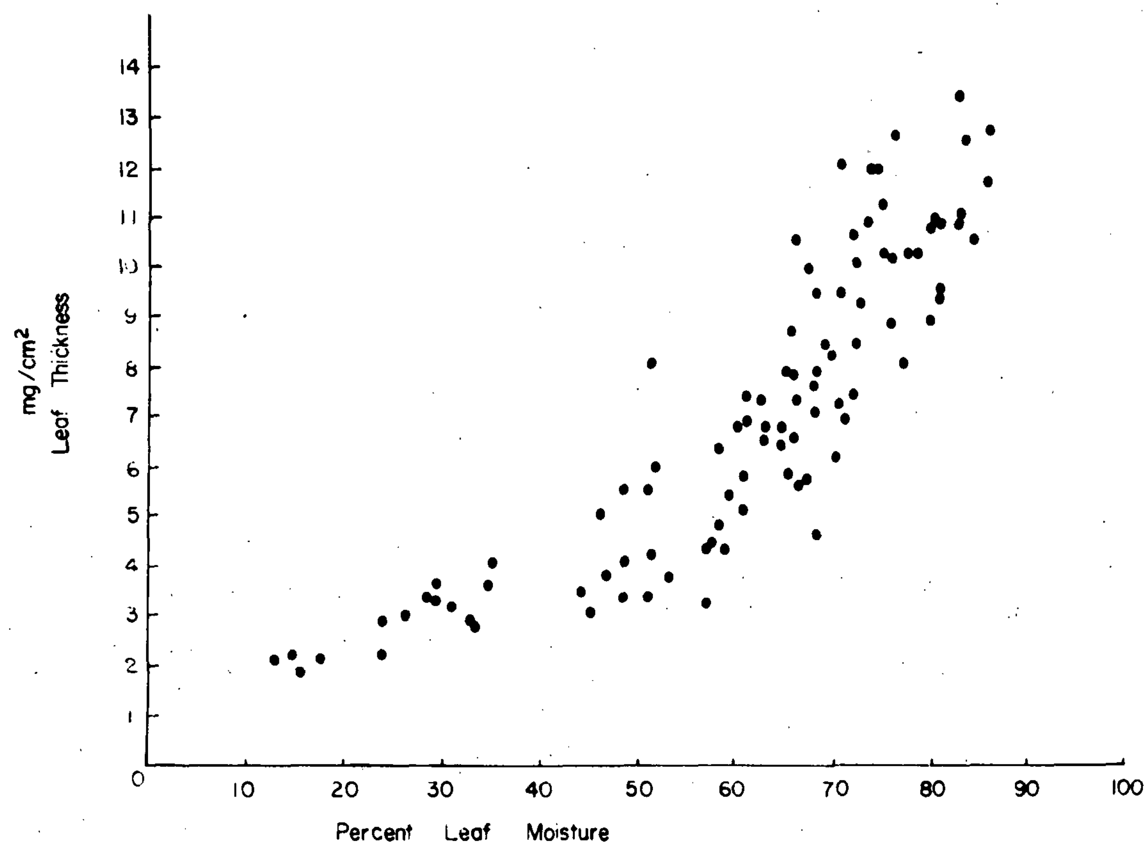

Figure 5. The relationship between leaf thickness and leaf moisture content for all leaves and treatments.